REMARKS/ARGUMENTS

Claims 1 and 3-29 were pending in the above-identified application. Claims 4-7, 10-12, 16, and 24-29 have previously been withdrawn from further consideration as being drawn to a non-elected invention. Claims 1, 3, 8, 9, 13-15, and 17-23 have been acted upon by the Examiner. In light of the remarks and arguments set forth below, Applicants respectfully request reconsideration of the application.

Rejections Under U.S.C. § 103:

Claims 1, 3, 8, 9, 13, 14, 17 and 18 remain rejected under 35 U.S.C. § 103(a) as being unpatentable over Matera *et al.*, 2000, in view of Bernard *et al.*, *Hematol. Cell Ther.* 40:17-26, 1998. In particular, as set forth previously the Examiner alleges that Matera *et al.* teach a method of differentiating dendritic cells comprising providing a population of peripheral blood monocytes that have been selected by magnetic sorting (*i.e.*, alleged to be non-activated), and contacting the monocytes with GM-CSF in the absence of additional cytokines citing to page 30 and 31 in particular. Matera *et al.* is also alleged by the Examiner to teach culturing in a serum free medium and to teach that the dendritic cells generated by culture with GM-CSF alone express CD1a. The Examiner has also alleged that the monocytic dendritic cell precursors of Matera *et al.* are the same as those produced by tangential flow filtration. Matera *et al.* is acknowledged by the Examiner not to teach a low avidity culture vessel comprising PFTE.

Bernard *et al.* is alleged by the Examiner to teach a method to generate dendritic cells from purified blood monocytes by culturing in a TEFLON™ (comprising PFTE) bag. Furthermore, the Examiner has alleged that Bernard *et al.* teaches that the method meets good laboratory practice (GLP) procedures necessary for the clinical use of dendritic cells. Therefore, the Examiner believes that it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to make the dendritic cells taught by Matera *et al.*, using the TEFLON™ culture vessel, as taught by Bernard *et al.*. Motivation for the ordinary artisan at the time the invention was made would have been provided since Bernard teaches that this

method is useful for clinical purposes, since it involves the large scale differentiation of dendritic cells in a culture system that meets GLP procedures. Moreover, one of ordinary skill in the art would have a reasonable expectation of success.

The Examiner has considered Applicants prior arguments and does not consider them persuasive. In particular, the Examiner notes that Applicants have argued that Matera *et al.* disclose that the monocytes are selected by magnetic sorting with a CD14 specific antibody, and that as evidence by Schutt *et al.*, CD14 specific antibodies can activate monocytes. The Examiner also notes that the term "non-activated" is not specifically defined in the specification and therefore "might encompass" a wide range of conditions. As an example of "non-activated", the Examiner provides a monocyte that is treated under conditions under which proliferation is not induced as in the case of CD14 positive selected cells. In addition, the Examiner has noted that Applicants disclosed in the specification a "non-activated" monocyte encompasses those positively selected with anti-CD14 antibodies. Further, the Examiner believes that the resulting dendritic cells produced by the method of Matera *et al.* are identical to those of the instant claims because they have decreased expression of CD14 and increased expression of CD1a, citing page 31).

Furthermore, the Examiner has alleged that Bernard *et al.* teaches that monocytes can be isolated by aphaeresis, which the Examiner characterizes as a "non-activating" method. It is the position of the Examiner that it would have been obvious to use aphaeresis as the method of monocyte isolation since selecting among the various methods of monocyte isolation would involve choosing among a finite number of predicable options which could be pursued with a reasonable expectation of success.

Applicants respectfully disagree with the rejection of claims 1, 3, 8, 9, 13, 14, 17 and 18 as being unpatentable under 35 U.S.C. § 103(a) over Matera *et al.*, in view of Bernard *et al.* Matera *et al.* is alleged by the Examiner to teach a method of differentiating dendritic cells comprising providing a population of peripheral blood monocytes that have been selected by magnetic sorting (a non-activating method) and contacting the non-activated monocytes with

GM-CSF in the absence of additional cytokines. To the contrary, Matera *et al.* disclose a method for differentiating a population of peripheral blood monocytes that have been selected by magnetic sorting and contacting with various agents. Applicants respectfully note that contact of monocytes with a CD14 specific antibody activates the monocytes. See for example, Schutt *et al.*, *Immunol. Lett.* 4:321-327, 1988, entitled "Human monocyte activation induced by an anti-CD14 monoclonal antibody". (Abstract attached). As such, Matera does not teach or suggest a method directed to non-activated monocytic dendritic cell precursor differentiation. Further, Matera *et al.* do not appear to disclose results that particularly relate to cells isolated using CD14 selection. The results section specifically relates to "adherent cells, cytokine driven under serum free conditions. (See, first line of the RESULTS section). As such, Applicants do not see that Matera *et al.* disclose a method that provides the same result as set forth in the present claims.

As to the specification lacking a specific definition of "non-activated". Applicants note that "non-activation" as used in the context of dendritic cell biology is well known to the skilled artisan. "Activation" as used in the present context is known to encompass the physiological steps that result in mature dendritic cells capable of inducing a T cell response. As such, adherence of monocytes to a surface is known to "activate" the monocytes to differentiate and mature to form "activated dendritic cells". See page 4 of the specification as filed. Sallusto *et al.* and others found that adhered monocytes cultured in the presence of GM-CSF and IL-4 would only differentiate into immature dendritic cells and would not continue maturation to form mature activated dendritic cells. Applicants found that if monocytic dendritic precursor cells could be obtained in a manner that prevented activation, IL-4 or other cytokines were not required to prevent maturation to mature dendritic cells. With the teachings provided by Applicants the skilled artisan could now modify antibody selection methods using anti-CD14 antibody such that activation does not occur. As such, the teachings in the specification are not inconsistent with the arguments against Matera *et al.* provided above.

Bernard *et al.* teaches the isolation of mononuclear cells from an aphaeresis (cytapheresis) method established for the collection of platelets. Human mononuclear cells were isolated by a centrifugation process using Ficoll Paque and further process in a counter current

centrifuge and isolated using a specific solution (TS 745 solution. The "purified monocytes" were cultured as described at the top of page 19, left column and culture of the cells in the presence of GM-CSF alone in Teflon bags resulted in the production of macrophage. Only culture of the isolated monocytes in the presence of GM-CSF and IL-4 resulted in immature DCs. This result is similar to that obtained by many skilled artisans at the time the present applications was filed. As such, given the teachings of Applicants' it must be concluded that the monocytes of Bernard *et al.* were activated during processing contrary to the allegations of the Examiner that "aphaeresis is a non-activating" process. The method of Bernard *et al.* is therefore not the same as, nor does it suggest the method of the present claims. Bernard *et al.* would actually appear to teach away from the method of the present claims.

Matera *et al.* therefore do not describe or suggest a method as disclosed and claimed in the present application. As both Matera *et al.* and Bernard *et al.* teach methods involving the differentiation of activated monocytes, there is no disclosure in either reference when considered alone or in combination that suggests or teaches the present invention. The Examiner is respectfully requested to therefore reconsider and withdraw the present rejection.

Claims 19-23 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Matera *et al.* and Bernard *et al.*, as applied to claims 1, 3, 8, 9, 13, 14, 17 and 18 above, in further in view of Bosch *et al.*, 2001 (of record). The teachings of Matera *et al.* and Bernard *et al.* are described above. The Examiner has acknowledged that they not teach generating maturing the dendritic cells with IFNγ and BCG. Bosch *et al.* is alleged by the Examiner to teach that dendritic cells can be matured with a combination of INFγ and BCG (*i.e.*, a bacterial antigen). Additionally, Bosch *et al.* is alleged by the Examiner to teach that maturation with IFNγ and BCG results in a dendritic cell population that can induce an immune response against a tumor antigen in cancer patients.

Therefore, the Examiner has asserted that it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to make a dendritic cell, as taught by Matera *et al.* and Bernard *et al.*, followed by maturation with BCG and IFNγ as taught

by Bosch *et al.*. The Examiner has asserted that the ordinary artisan would have been motivated to do so, since Bosch *et al.* teach that IFNγ and BCG are extremely potent maturation agents that result in a dendritic cell population that can induce an immune response against a tumor antigen in cancer patients. Moreover, the Examiner believes that one of ordinary skill in the art would have a reasonable expectation of success, since Bosch *et al.* teaches the effectiveness of these techniques in the generation of dendritic cells.

Applicants respectfully disagree with the rejection of claims 19-23 as being unpatentable over Matera *et al.* and Bernard *et al.*, as applied to claims 1, 3, 8, 9, 13, 14, 17 and 18 above, in further in view of Bosch *et al.*, 2001. Matera *et al.* and Bernard *et al.* are discussed above. Both references describe methods directed to the differentiation of activated monocytes and not non-activated monocytic dendritic cell precursors as set forth in the instant claims. In addition, both Matera and Bernard require GM-CSF in combination with some other agent, such as either the cytokine IL-4 or the hematopoietic protein prolactin, for the differentiation of the isolated activated monocytes to form dendritic like cells. Applicants note that Matera *et al.* define prolactin as a member of the cytokine family that has been demonstrated to modulate the T cell antigen receptor expression and phosphorylate the kinases activated by T cell receptor ligation.

Bosch *et al.* disclose a method for the production of immature dendritic cells that uses serum free conditions, but provides no additional details such as whether the method utilizes non-activated monocytic dendritic cell precursors or whether GM-CSF and IL-4 were used. Bosch *et al.* as set forth by the Examiner disclose the maturation of dendritic cells in the presence of BCG and IFNγ for the induction of an antigen specific cytotoxic T cell response. But, the references when considered alone or in any combination fail to teach a method for the production of immature dendritic cells from non-activated monocytic dendritic cell precursors in the presence of GM-CSF and without additional cytokines as presently claimed.

As such, Applicants respectfully request the Examiner to reconsider and withdraw the rejection of claims 19-23 as being unpatentable over Matera *et al.* and Bernard *et al.* in further view of Bosch *et al.*

Claim 15 remains rejected under 35 U.S.C. § 103(a) as being unpatentable over Matera *et al.* and Bernard *et al.* as applied to claims 1, 3, 8, 9, 13, 14, 17 and 18 above, and further in view of Lewalle *et al.*, 2000 (of record). The alleged teachings of Matera *et al.* and Bernard *et al.* are described above. The Examiner has acknowledged they do not teach using a cryopreserved cell population to generate dendritic cells. Lewalle *et al.* is alleged by the Examiner to teach the generation of dendritic cells from frozen peripheral blood mononuclear cells. Furthermore, Lewalle *et al.* is alleged by the Examiner to teach that many clinical protocols are based on sequential injections of dendritic cells, and therefore it would be of practical importance to have frozen aliquots of the same peripheral blood mononuclear cells for these purposes. Based on these allegations and assertions that Examiner believes that it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to make the dendritic cell taught by Matera *et al.* and Bernard *et al.*, using frozen peripheral blood mononuclear cells, as taught by Lewalle *et al.*. Motivation for the ordinary artisan at the time the invention was made is alleged by the Examiner to be based on Lewalle *et al.* teaching that many clinical protocols are based on sequential injections of dendritic cells, and the practical importance to have frozen aliquots of the same peripheral blood mononuclear cells for these purposes. Furthermore, the Examiner believes that the ordinary artisan would have had a reasonable expectation of success since Lewalle teaches that dendritic cells derived from frozen peripheral blood mononuclear cells retain their functional capacity.

Applicants respectfully disagree with the rejection of claim 15 as being unpatentable over Matera *et al.* and Bernard *et al.* as applied to claims 1, 3, 8, 9, 13, 14, 17 and 18 above, and further in view of Lewalle *et al.*, 2000. As above, Matera *et al.* and Bernard *et al.* when considered either alone or in any combination fail to teach the methods of the present invention. Lewalle *et al.* also does not disclose or suggest a method for differentiating non-activated human monocytic dendritic cell precursors into immature dendritic cells having

decreased CD14 and having increased CD1a on the cell surface, comprising: providing a cell population comprising non-activated human monocytic dendritic cell precursors, and contacting the non-activated monocytic dendritic cell precursors in a culture vessel with a dendritic cell culture media supplemented with granulocyte-macrophage colony stimulating factor in the absence of additional cytokines under conditions that do not activate the monocytic dendritic cell precursors. As such, Lewalle *et al.* does not provide any feature of the invention that is not disclosed in Matera *et al.* and/or Bernard *et al.* Lewalle *et al.* is directed to the generation of dendritic cells from frozen peripheral blood mononuclear cells. Such a disclosure does not teach or suggest any method for the production of immature dendritic cells from non-activated monocytic dendritic cell precursors. The references when considered either alone or in any combination also do not provide or suggest that the skilled artisan would have any reasonable expectation of success in developing such a method. Therefore, Applicants respectfully request the Examiner reconsider and withdraw the rejection of claim 15 as being unpatentable over Matera *et al.* and Bernard *et al.*, and further in view of Lewalle *et al.*

Applicants respectfully request the Examiner to reconsider and withdraw the rejection of claim 15 as being unpatentable over Matera *et al.* and Bernard *et al.* as applied to claims 1, 3, 8, 9, 13, 14, 17 and 18 above, and further in view of Lewalle *et al.*, 2000 in view of the above remarks.

New Grounds of Rejection

Rejections Under 35 U.S.C. § 102:

Claims 1, 13, 14, 17, and 18 stand rejection under 35 U.S.C. § 102(b) as being anticipated by Matera *et al.* (*supra*). In particular, the Examiner alleges that Matera *et al.* teach a method of differentiating dendritic cells comprising providing a population of peripheral blood monocytes that have been selected by magnetic sorting and contacting the monocytes with GM-CSF in the absence of additional cytokines. Matera *et al.* is also alleged by the Examiner to teach culturing in a serum free medium and to teach that the dendritic cells generated by culture with GM-CSF alone express CD1a. The Examiner has also noted that the instant claims are

drawn to a method of differentiating dendritic cells employing a dendritic cell precursor and therefore the method by which the monocytic precursor is produced does not carry patentable weight in the absence of a structural difference. It is further alleged by the Examiner that the monocytic dendritic cell precursors of Matera *et al.* are the same as those produced by tangential flow filtration and that the culture conditions comprising culture with GM-CSF taught by Matera *et al.* can be considered "non-activating" since they do not result in the progression of a fully mature dendritic cells.

Applicants must disagree with the rejection of claims 1, 13, 14, 17, and 18 as being anticipated by Matera *et al.* In particular, although Matera *et al.* state on page 30, right column, that in some experiments cultures were started with a purified population of CD14 positive cells selected with magnetic microbeads, Applicants can find no results specifically associated with these cells. The results summarized on page 31 and depicted in the Figures are said to be with adherent cells, cytokine driven under serum free conditions. In addition, the "dendritic cells" obtained when cultured with GM-CSF alone are not the same as those encompassed by the instant claims. As summarized by the authors Gm-CSF and IL-4 strongly enhanced CD1a expression as compared with GM-CSF alone. Further, GM-CSF alone was found to be "very effective at inducing macrophage-like cells". See page 32, right column, line 3-4 or the section entitled "Morphology of cytokine-derived DC". As such, the methods of Matera *et al.* do not produce the same dendritic cell precursors as the instant claims. The cells are structurally different and the methods of Matera *et al.* can not be considered non-activating.

Claims 1, 14, 17 and 18 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Kasinrerk *et al.*, *J. Immunol.* 150:579-584, 1993. The Examiner alleges that Kasinrerk *et al.* teach a method of differentiating monocytes comprising providing a population of peripheral blood monocytes that have been selected by density gradient centrifugation and negative selection (alleged by the Examiner to be non-activating), and contacting said monocytes with GM-CSF in the absence of additional cytokines. In addition, Kasinrerk *et al.* is alleged by the Examiner to teach that the resulting cells have an increased expression of CD1a and that the resulting cells have a decrease in the expression of CD14. As such, the Examiner has concluded

that the cells of Kasinrerk *et al.* are the same as those of the instant claims. Furthermore, the Examiner has alleged that the culture of the monocytes with GM-CSF alone in the absence of other stimulating cytokines , as taught by Kasinrerk *et al.*, can be considered non-activating conditions. Still further, the Examiner alleges that although the monocytes of Kasinrerk *et al.* have been purified using a different process than tangential flow filtration, the monocytes nevertheless are structurally the same as the monocytic precursor cells of the instant claims.

Applicants must disagree with the rejection of claims 1, 14, 17 and 18 as being anticipated by Kasinrerk *et al.* In particular, the Examiner has alleged that the population of peripheral blood monocytes obtained by Kasinrerk *et al.* are non-activated monocytic dendritic precursor cells because they have been isolated by density gradient centrifugation and because the isolated monocytes were shown to express CD1a when contacted with GM-CSF alone. Applicants do not agree with this conclusion. The monocytic cell population of Kasinrerk *et al.* was isolated by a method that included density gradient centrifugation, incubation with neuraminidase-treated sheep red blood cells, and negative selection using anti-CD19 and CD56. The isolated monocytes where only assayed for the expression of CD1a, CD1b and CD1c subsequent to culture for up to three days with GM-CSF alone. As such, it is not possible for the Examiner to conclude that the monocytes obtained by the method of Kasinrerk *et al.* were non-activated. There is also no data provided regarding the presence of CD14. As such, Kasinrerk *et al.* does not anticipate the method of the instant claims.

Applicants respectfully request that the Examiner reconsider and withdraw the rejection of claim 1, 14, 17 and 18 as being anticipated by Kasinrerk *et al.* in view of the above remarks.

Rejection Under 35 U.S.C. § 103:

Claims 1, 3, 8, 9, 13, 14, and 17 through 23 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Kasinrerk *et al.* (*supra*), in view of EP 0205387. In particular, the Examiner alleges that Kasinrerk *t al* teach as described above, but do not teach serum free

medium, culturing in low avidity culture vessel, and contacting the cells with an antigen, or with BCG and IFN-gamma. EP 0205387 is alleged by the Examiner to teach a method for culturing monocytes in serum free medium in a low avidity culture flask. In addition, EP 0205387 teach using a TEFLON™ vessel and teaching that monocytes can be activated for therapeutic purposes by contacting the cells with antigen, or with compounds including IFN-gamma and BCG. Further, the Examiner alleges that the method provides precisely defined consistent and uniform conditions for obtaining large numbers of monocytes useful for experimental, pharmaceutical, or therapeutic purposes. It is the Examiner's belief therefore, that it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to use the serum free low avidity culture system of EP 0205387, in the method of differentiating monocytes taught by Kasinrerk *et al.* The Examiner alleges that the ordinary artisan would have been motivated to do so, since EP 0205387 teaches that the method provides precisely defined consistent and uniform conditions for obtaining large numbers of monocytes useful for experimental, pharmaceutical, or therapeutic purposes. Additionally, the Examiner alleges that it would have been obvious to further treat the differentiated monocytes of Kasinrerk *et al.* with antigen, BCG and IFN-gamma, since EP 0205387 teaches that such treatments enhance activation of monocytes for therapeutic purposes.

Applicants must respectfully disagree with the rejection of claims 1, 3, 8, 9, 13, 14, and 17 through 23 as being unpatentable over Kasinrerk *et al.* in view of EP 0205387. Kasinrerk *et al.* is discussed above. EP 0205387 is directed to methods of culturing monocytes in a low avidity culture system without serum. There is no disclosure or suggestion of culturing the isolated monocytes, whether activated or non-activated in GM-CSF alone to obtain immature dendritic cells. EP 0205387 teaches the contacting of monocytes with BCG or IFN-gamma, not immature dendritic cells. It should be noted that, for example, Bernard *et al.* (*supra*) and Sallusto and Lanzavecchia, *J. Exp. Med.* 179:1109-1118, 1994 (previously cited by the Examiner) disclose that culture of "monocytes" with GM-CSF alone results in either mature dendritic cells or macrophage. Bernard *et al.* cultured "monocytes" in TEFLON bags in the presence of GM-CSF alone and obtained macrophage. As such, it would not be obvious to one

of skill in the art to combine the teaching of Kainrerk *et al.* and EP 0205387 to obtain the methods of the instant claims.

It is respectfully requested that the Examiner reconsider and withdraw the rejection of claims 1, 3, 8, 9, 13, 14, and 17 through 23 as being unpatentable over Kasinrerk *et al.* (*supra*), in view of EP 0205387 in view of the remarks above.

Claims 1, 14, 15, 17, and 18 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Kasinrerk *et al.* (*supra*), in view of Stevenson *et al.*, *J. Leuk. Biol.* 36:521-531, 1984. In particular, the Examiner directs Applicants to the alleged teachings of Kasinrerk *et al.* above and notes that Kasinrerk *et al.* do not teach a cryopreserved cell population. Stevenson *et al.* is alleged by the Examiner to teach that cryopreserved monocytes are suitable for culture and that cryopreserved monocytes function similarly to fresh cells, that they are more convenient, and reproducible for use in *in vitro* assays. As such, the Examiner alleges that it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to use the cryopreserved monocytes of Stevenson *et al.* in the method of differentiating monocytes taught by Kasinrerk *et al.* The Examiner believes that the ordinary artisan would have been motivated to do so because Stevenson *et al.* teaches that cryopreserved monocytes function similarly to fresh cells, and that they are more convenient and reproducible for use in *in vitro* assays.

Applicants respectfully disagree with the rejection of claims 1, 14, 15, 17 and 18 as being unpatentable over Kasinrerk *et al.* (*supra*) in view of Stevenson *et al.* Kasinrerk *et al.* is discussed above and does not teach the method of the present invention. In addition to Kasinrerk *et al.* failing to teach any more than the induction of CD1a by GM-CSF, other teachings at the time of the present invention suggested that the culture of monocytes in the presence of GM-CSF alone would result in either mature dendritic cells or macrophage. Stevenson *et al.* does not teach or suggest a method for differentiating human monocytic dendritic cell precursors into immature dendritic cells of any type. Stevenson *et al.* merely teach cryopreservation of isolated monocytes. The monocytes when thawed showed some similarities in function in certain *in vitro*

assays. None of the disclosed assays was related to the methods of the instant claims. In addition, the is no indication whether cryopreservation activated the monocytes to form macrophage or some other myeloid cells type. As such, there is no *prima facie* reason for the skilled artisan to combine the teachings of Kasinrerk *et al.* with Stevenson *et al.* to obtain the method of the present claims. In fact, the combination of the references would, at most, merely test whether the monocytes of Kasinrerk *et al.* might maintain the ability to produce CD1a when cultured with GM-CSF. There is no suggestion or disclosure that the cells produced by the method would be immature dendritic cells.

The Examiner is respectfully requested to reconsider and withdraw the rejection of claims 1, 14, 15, 17 and 18 as being unpatentable over Kasinrerk *et al.* (*supra*) in view of Stevenson *et al.* in view of the remarks above.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested. If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 206-467-9600.

Respectfully submitted,

Dated: 5 February 2010 By: Brian W. Poor

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